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Absorption, Distribution and Milk Secretion of Radionuclides by the Dairy Cow - V. Radiotungsten

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16. ABSTRACT

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The average per cent of administered dose secreted per liter of milk at peak activity was 0.01 and 0.1 for the groups receiving oral and I.V. doses, respectively. During the 84-hr period after dosing, 79% of the orally administered dose was recovered, with 64% recovered in the feces, 14.6% in urine and 0.4% in milk. In the same time period, 68% of the intravenous dose was recovered with 65% in urine, 2% in the milk, and less than 1% in feces.

Tissue distribution results indicate the principal sites of short-term radiotungsten deposition were skin, liver and soft tissue. Long-term retention sites in mature cows were bone, muscle and skin. Long-term retention sites in calves were bone, adrenal, skin and spleen.

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Milk	Distribution and transport	07B
Cows		07C
Metabolism	Exposure/dose assessment	07E
Radioactive Isotopes		18B
Radiation Dosage		

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ABSORPTION, DISTRIBUTION AND MILK SECRETION OF RADIONUCLIDES BY THE DAIRY COW—V. RADIOTUNGSTEN*

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Abstract—In a study conducted in 1969 and 1970 lactating cows were given oral or intravenous (I.V.) doses of radiotungsten. Blood, milk, urine and feces were collected and analyzed to measure transfer rate and quantity of tungsten eliminated over an extended period during and after exposure. Several lactating and nonlactating cows were sacrificed to measure tissue distribution of radiotungsten and several calves were sacrificed following oral administration of radiotungsten to determine the distribution in rapidly growing young.

The average per cent of administered dose secreted per liter of milk at peak activity was 0.01 and 0.1 for the groups receiving oral and I.V. doses, respectively. During the 84-hr period after dosing, 79% of the orally administered dose was recovered, with 64% recovered in the feces, 14.6% in urine and 0.4% in milk. In the same time period, 68% of the intravenous dose was recovered with 65% in urine, 2% in the milk and less than 1% in feces.

Tissue distribution results indicate the principal sites of short-term radiotungsten deposition were skin, liver and soft tissue. Long-term retention sites in mature cows were bone, muscle and skin. Long-term retention sites in calves were bone, adrenal, skin and spleen.

INTRODUCTION

As the human young are dependent on milk as a staple food source, milk-borne radionuclides may be a significant source of exposure for this critical segment of the population. Studies with dairy cows designed to measure the milk transfer of various radionuclides provide important information for estimating the potential hazard from the milk link in man's nutrient chain. Of equal importance to the rapidly growing teenager and young adult is the protein derived from beef cattle. A study of the distribution and concentration of radionuclides in the tissues of beef cattle is a necessity if the populace is to be properly protected in the event of an accidental release of radionuclides.

Thermonuclear explosions may produce a number of radionuclides by neutron activation. In some instances, i.e. Project Schooner of 8 December 1968, a nuclear cratering experiment at the Nevada Test Site, the predominant activity in fallout at early times was tungsten-187 (La63; Te70). The predominant activity after several months may be due to other tungsten isotopes, i.e. tungsten-181 and tungsten-185 (Es65; SRHL69). As little was known of the transport of tungsten isotopes to milk or concentration in beef products, this study was conducted in 1969 and 1970 to measure the fraction secreted in milk and to evaluate the potential significance of the milk and meat links in man's nutrient chain.

PROCEDURE

(a) *Milk transfer and excretion*

The dairy cows used in the study were selected from the 20-cow milking herd of purebred Holsteins maintained at the Nevada

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† Formerly National Environmental Research Center.

Test Site by the U.S. Environmental Protection Agency's Environmental Monitoring and Support Laboratory, Las Vegas. The cows used in the experiments ranged from 3 to 12 yr of age and were 15–200 days into the current lactation period. Daily milk production ranged from 12 to 36 kg of milk. Throughout the study, the cows were given free access to water and alfalfa hay supplemented with approx 5 kg of a pelleted commercial dairy concentrate containing 16% protein. The calves used to study the tissue distribution of tungsten ranged from 1 to 5 months of age. The calves had free access to hay in addition to twice daily feedings of milk.

During the major portion of the study, the cows were confined in metabolism stalls. These stalls facilitate total collection of feces and urine, permit access to either side for application of the milking machine, and are unrestrictive enough to allow the cows to lie down. Feces were collected in a grid-covered pan at the rear of the stall. An in-dwelling catheter inserted into the bladder of each cow and connected to a 20-l. bottle by polyethylene tubing was used for urine collection. Milking was accomplished with a milking machine and blood samples were taken periodically by jugular venipuncture. After the cows had been catheterized and allowed to become accustomed for about 4 hr to the limited confinement of the metabolism stalls, samples of milk, feces, urine and blood were taken for background activity measurements.

The major portion of this study, concerned with milk transfer of radioactive tungsten, was conducted in three consecutive experiments using four lactating cows in each. The tungsten-187 ($t_{1/2} = 24$ hr) was obtained as tungstate in a 3 N sodium hydroxide solution. The specific activity was 44.5 mCi/mg W and the purity >98%, with tungsten-181 being the major impurity. The tungsten-181 ($T_{1/2} =$ 140 days) was obtained in a 0.5 N HCl and 0.1 N HF solution with a specific activity of 1.26 mCi/mg W and a purity of >99%.

For the first experiment a single oral dose (1 mCi) of tungsten-187 was administered to each cow. The solution was contained in a ½-ounce gelatin capsule which was placed inside a 1½-ounce capsule and administered with a balling gun. Double encapsulation was used to minimize the possibility of capsule rupture during dosing. Total urine and fecal accumulations were collected every 6 hr for the duration of the cows' confinement in the stalls (96 hr). The individual accumulations were weighed, mixed thoroughly and 400-ml aliquots placed in polyethylene containers for gamma counting. Blood samples and total milk accumulations were collected every 6 hr for the first 24 hr, and at each 12-hr interval thereafter. A 400-ml aliquot from each milking was gamma counted. The blood samples, approx 40 ml, were gamma counted as whole blood, then centrifuged and the serum portion counted separately. All samples were counted using NaI(Tl) crystals connected to single-channel analyzers. Samples were counted for up to 40 min and sample collection was discontinued when the counting error exceeded 30%. The doses, aliquoted by volume measurements of known dilutions, were verified prior to administration by gamma counting the capsules.

For the second experiment, four cows were each given a total of 512 μCi of tungsten-181 solution in 14 equal oral doses over a 7-day period. This was administered at 12-hr intervals immediately following sample collection. Dosing and sample collection and counting procedures were carried out as described in the first phase of the study. The animals remained in the metabolism stalls for 203 hr, and were then transferred to outside holding pens. After removal of the animals from the stalls, only milk samples were collected for the remainder of the study. When the milk values reached the lower limit of counting sensitivity for 400-ml counting containers, a 3.5-l. Marinelli beaker was used. When the lower limit of the Marinelli beaker counting geometry was reached, the milk was passed through Dowex 2X-8(Cl^-) ion-exchange resin to remove the tungsten, and the resin-bound activity was counted.

The third experiment of this series consisted of a single intravenous injection of 1 mCi tungsten-187, as tungstate in isotonic saline solution, into the jugular veins of four cows with subsequent sample collection over a 240-hr period for milk and 131 hr for urine and

fecal excretions. Conditions were kept the same as those in the other experiments of the study.

(b) *Tissue distribution*

Several experiments were carried out to determine tissue distribution of radiotungsten. A non-lactating cow was placed in a metabolism stall and administered tungsten-181 orally twice daily for 3 consecutive days (254 μCi total). Urine and fecal excretions were collected as described previously. Three and one-half days after initial dosing, the cow was sacrificed; one lactating cow from the multi-dose tungsten-181 group was also sacrificed 12 days after dosing ceased in order to compare longer term tissue distribution.

To determine tissue distribution in calves, one Hereford calf (172 kg) was given tungsten-181 orally twice daily for 3 days and sacrificed. In addition, two Holstein calves (weighing 56.2 and 268 kg) were fed milk twice daily from the cows in the multi-dose study and one was sacrificed at 3.5 days and the other 27.5 days after dosing ceased. Since the daily activity in the milk fed to the calves followed the secretion curve for tungsten in the lactating cow, this would simulate a single contamination of forage. Selected tissues were removed and weighed. Estimates of the total weights were calculated for muscle, blood, bone, fat and hide (NAS68). The bones selected to be representative of the skeleton were the hock, femur, rib and sternum. For the purpose of determining the total percentage of radiotungsten in the bone tissue of the entire skeleton, the bone values were averaged. The bone marrow was included with the bone. All tissue samples were placed in containers of appropriate size before counting as described previously.

(c) *Binding properties of tungsten in milk*

A 3-l. milk sample was collected from each of the study cows. On a set time schedule, duplicate 200-ml aliquots from these samples were passed through an anion exchange column, under conditions generally used for removal of radioiodine (Po65) and the resin subsequently counted. The objectives of this portion of the study to determine the binding properties of radiotungsten in milk were (1) to determine if binding to the organic fraction of the milk changed as absorbed radiotungsten was mobilized and incorporated into milk so as to ascertain whether its availability to young animals could change with time and (2) to determine whether binding of radiotungsten in milk increased as a function of time after sampling so as to determine the validity of the ion-exchange method of analysis. It was assumed that the tungsten which was not absorbed on the resin was associated with organic compounds in the milk. As a control, *in vitro* experiments were carried out by adding radiotungsten to milk samples from four other cows and duplicate aliquots processed in the same manner as the *in vivo* labeled samples described above.

RESULTS

(a) *Milk transfer and excretion*

The results are shown in the appended tables and figures. All data were decay corrected to initial time of administration. The biological half-life and effective half-life for each compartment were determined by least squares fit to the data.

Figure 1 shows the results of the single dose tungsten-187 study. The peak activity in milk occurred within 24 hr after administration of the radiotungsten and was 0.0128% of the administered dose per liter. The biological half-time of tungsten-187, as measured in milk, was found to be 18.6 hr. The effective half-time was 10.2 hr as calculated from the activity in milk collected after the peak concentration was reached.

Total activity recovered in urine, feces and milk was 50% in 30 hr, 75% in 60 hr, and 80% in 96 hr. In 96 hr, 81% was recovered in the feces, 18.5% in the urine, and 0.5% in the milk.

As shown in Fig. 2, the biological half-time of intravenously administered tungsten-187, as measured in milk, was found to be 7.3 hr over the first 48 hr, followed by a longer biological half-time of 31.4 hr. The peak concentration in the milk occurred during the first 6 hr after dosing and was approx 0.118% of the administered dose per liter. Total activity

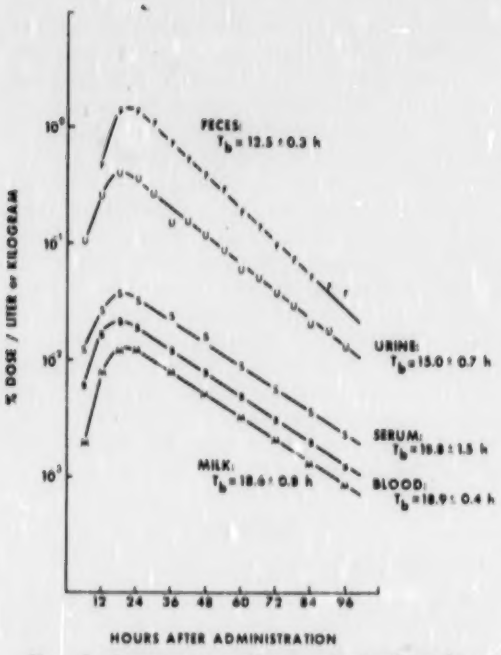
% DOSE / LITER or KILOGRAM
FECES:
T_b = 12.5 ± 0.3 h
URINE:
T_b = 15.0 ± 0.7 h
SERUM:
T_b = 16.8 ± 1.5 h
MILK:
T_b = 18.6 ± 0.8 h
BLOOD:
T_b = 18.9 ± 0.4 h
12 24 36 48 60 72 84 96
HOURS AFTER ADMINISTRATION

FIG. 1. Average excretion concentrations for tungsten-187 given as a single oral administration to four lactating dairy cows.

recovered in the urine, feces and milk was 60% in 12 hr and 68% in 92 hr. Of the amount excreted in 92 hr, 1% was excreted via the feces, 95.7% via the urine and 3% secreted via the milk.

Figure 3 illustrates the results of the multi-dose administration of tungsten-181. The peak activity in the milk was reached at 59 hr. The concentration of tungsten secreted in the milk remained relatively constant during dosing (biological half-time 112 ± 17 hr). After the end of dosing, the milk secretion curve indicated two different biological half-times (T_b). These were: $T_b = 21.7 \pm 0.6$ hr for 7 days following dosing and $T_b = 202 \pm 52$ hr for the remainder of the study (50 days).

During the 48 hr following the final administration of tungsten-181, 81.5% of the activity was present in the feces, 18.5% in the urine and 0.7% in the milk. These data are in good agreement with the data from the single oral dose experiment using tungsten-187. In this experiment, over the same time period, 81.5% of the activity was recovered in the feces, 17.9% in the urine and 0.5% in the milk.

The tungsten activity found in the formed elements of the blood and the serum is shown in Figs. 1–4. The results from the single oral administration show that the tungsten was concentrated mainly in the blood serum. The blood concentrations corresponded closely with those shown for the milk, indicating blood to milk equilibrium occurred rapidly after ingestion of radiotungsten and did not change during the 96 hr following administration. During multiple ingestion it appears that the radiotungsten is present in both the formed elements and serum of the blood. Tungsten concentrations in the whole blood correspond closely with the values shown in milk, whereas the serum values are much lower and show a shorter half-time during the

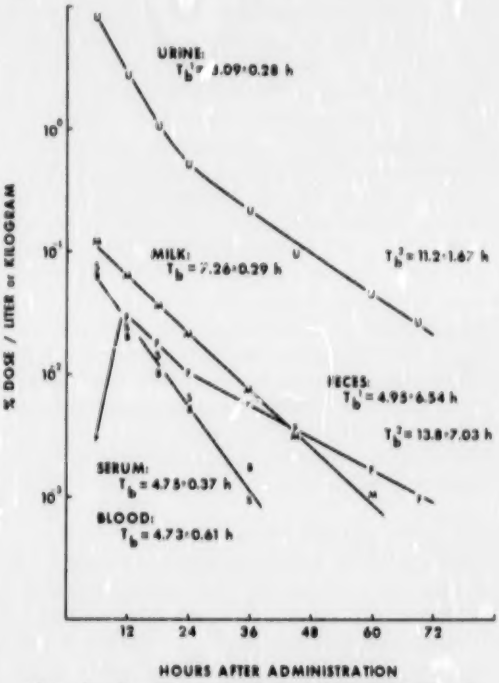
% DOSE / LITER or KILOGRAM
URINE:
T_b^1 = 3.09 ± 0.28 h
T_b^2 = 11.2 ± 1.67 h
MILK:
T_b = 7.26 ± 0.29 h
FECES:
T_b^1 = 4.95 ± 6.54 h
T_b^2 = 13.8 ± 7.03 h
SERUM:
T_b = 4.75 ± 0.37 h
BLOOD:
T_b = 4.73 ± 0.61 h
12 24 36 48 60 72
HOURS AFTER ADMINISTRATION

FIG. 2. Average excretion concentrations of tungsten-187 given as a single intravenous administration to four lactating dairy cows.

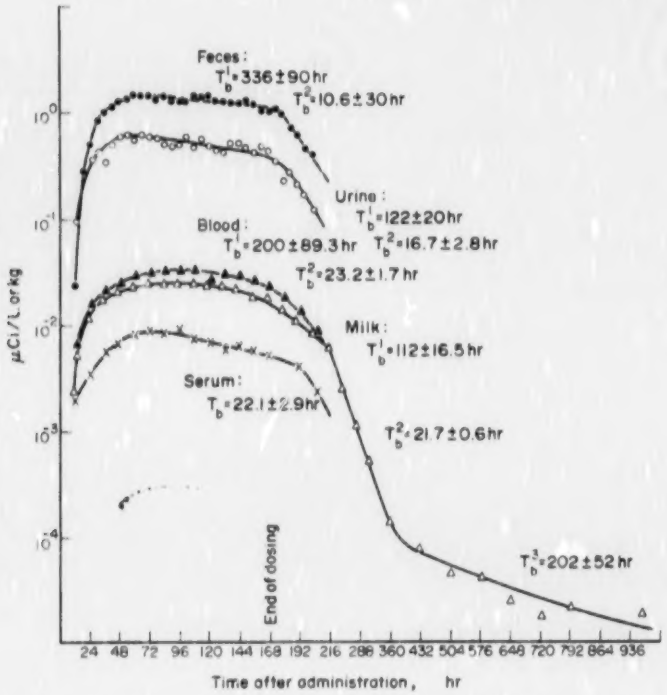

FIG. 3. Average excretion concentrations of tungsten-181 after multiple oral administration to four lactating dairy cows.

dosing period indicating that the radiotungsten may have been incorporated into the formed elements of the blood shortly after absorption. The tungsten activity in the blood of a nonlactating cow receiving multiple oral administrations of radiotungsten shows that the concentration was in the serum. The activity in the blood of cows receiving radiotungsten intravenously dropped rapidly, showing less correspondence with the amounts secreted into the milk than did the blood of cows receiving radiotungsten orally.

(b) *Tissue distribution*

Figure 4 shows the tungsten concentration in blood, serum, urine and feces from a nonlacting dairy cow administered tungsten-181 orally for 3 days. The non-lactating cow was sacrificed 86 hr after the initial oral administration at which time 56% of the dose had been excreted. Of this amount, 76.7% had been excreted in the feces and 23.3% in the urine. In 83 hr after initial dosing, the lactating cows receiving multiple doses had excreted 74.4% in the feces and 24.8% in the urine, indicating little difference in the metabolism of the radiotungsten by non-lactating animals.

Following sacrifice, 36% of the administered dose was recovered from the carcass of the non-lactating cow accounting for a total of 91% of the dose. Only 21% of the administered dose was recovered from the carcass of the calf dosed and sacrificed in the same manner as the cow. The distribution of radiotungsten in the tissue of the calf and non-lactating cow is shown in Table 1.

Results of the tissue distribution of tungsten-181 in a lactating cow sacrificed 18.5 days after initial dosing indicated little long-term deposition had occurred. At the time of sacrifice approx 99% of the administered dose

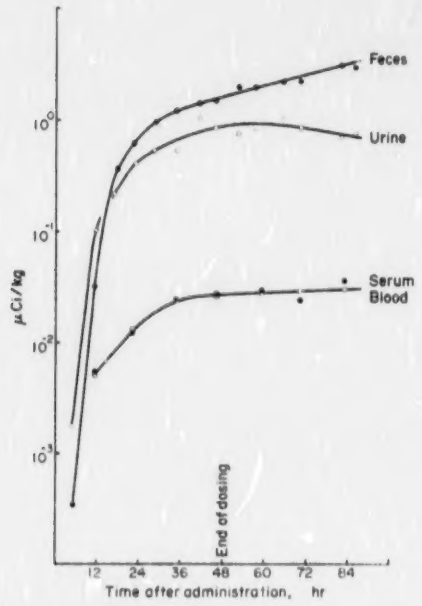

FIG. 4. Excretion concentration of tungsten-181 after multiple administration to one non-lactating dairy cow.

Table 1. Tissue distribution of radiotungsten in a calf and a non-lactating cow given multiple oral doses of tungsten-181

Tissue or compartment	Calf (%/kg)	Calf (%/organ)	Non-lactating cow (%/kg)	Non-lactating cow (%/organ)
Abomasal contents	2.10*	1.08	0.399	0.721
Abomasal tissue	0.026	0.013	0.011	0.022
Adrenal	0.056	<0.001	0.013	<0.001
Blood	0.021	0.228	0.008	0.351
Bone	0.105	2.35	0.004	0.386
Brain	0.002	<0.001	<0.001	<0.001
Eye	0.020	<0.001	0.043	<0.001
Fat	0.005	0.224	<0.001	0.730
Gall bladder w/bile	0.007	0.001	0.002	<0.001
Gonads	0.740	0.004	0.147	0.001
Heart	0.016	0.014	0.004	0.013
Kidney	0.100	0.041	0.042	0.075
Large intestine contents	3.27	2.94	0.726	9.72
Large intestine tissue	0.050	0.069	0.009	0.039
Liver	0.067	0.143	0.017	0.179
Lung	0.034	0.035	0.009	0.043
Muscle	0.003	0.171	<0.001	0.220
Omasal contents	0.225	0.986	0.798	6.07
Omasal tissue	0.030	0.028	0.012	0.081
Pancreas	0.020	0.005	0.007	0.005
Parotid	0.003	<0.001	0.005	0.002
Reticulum tissue	0.003	0.001	0.019	0.032
Rumen-reticulum contents	1.20	10.9	0.342	11.47
Rumen tissue	0.007	0.119	0.050	0.417
Skin w/hair	0.033	0.351	0.006	0.253
Small intestine contents	0.637	0.490	0.326	3.62
Small intestine tissue	0.052	0.121	0.018	0.155
Spleen	0.022	0.167	1.03	1.71
Thyroid	0.021	<0.001	0.008	<0.001
Total		20.5%		36.3%

* Values are expressed as per cent of administered activity and are decay corrected to time of administration.

Table 2. Tissue distribution of tungsten-181 in calves ingesting contaminated milk

Tissue or compartment	Fed 4.5 days (%/kg)	0.369 μCi total (%/organ)	Fed 27.5 days (%/kg)	2.25 μCi total (%/organ)
Abomasal contents	4.35*	6.07	<0.001	<0.001
Abomasal tissue	2.74	0.650	<0.001	<0.001
Adrenal	2.00	0.008	0.070	0.002
Blood	0.164	0.590	<0.001	<0.001
Bone	0.268	1.98	0.052	1.72
Brain	1.06	0.205	<0.001	<0.001
Eyes	0.296	0.032	<0.001	<0.001
Fat	0.160	2.29	<0.001	<0.001
Gall bladder w/bile	0.571	0.012	<0.001	<0.001
Gonads	0.647	0.011		
Hair	0.029		0.009	
Heart	1.65	0.642	<0.001	<0.001
Kidney	3.45	0.677	0.007	0.006
Large intestine contents	115.0	16.5	<0.001	0.003
Large intestine tissue	0.420	0.174	<0.001	<0.001
Liver	0.537	0.306	<0.001	0.001
Lung	0.392	0.285	<0.001	<0.001
Muscle	0.005	0.108	<0.001	<0.001
Omasal contents	1.05	0.046	<0.001	<0.001
Omasal tissue	0.432	0.016	0.033	0.053
Pancreas	<0.001	<0.001	<0.001	<0.001
Parotid	0.642	0.009	<0.001	<0.001
Reticulum tissue	<0.001	<0.001	<0.001	<0.001
Rumen-reticulum contents	0.153	0.289	0.007	0.024
Rumen tissue	2.24	0.533	<0.001	0.004
Skin w/hair	0.410	1.43	0.028	0.462
Small intestine contents	2.28	0.807	<0.001	<0.001
Small intestine tissue	0.941	1.04	0.001	0.003
Spleen	2.61	0.520	0.009	0.005
Thyroid	1.29	0.018	0.044	0.001
% of dose recovered		35.5		2.30

* Values are expressed as per cent of administered activity and are decay corrected to time of administration.

had been recovered in the milk, urine and feces, leaving a very small concentration in muscle, bone, adrenals, skin and liver.

Table 2 shows the distribution of radiotungsten in the tissues of calves fed milk from the cows ingesting tungsten-181. Based on total organ or compartment, the principal sites of short-term deposition were pelt (skin and hair), liver and soft tissue. Long-term retention in the mature cows was in bone, muscle and pelt. Long-term retention sites in the calves fed contaminated milk were bone, adrenals, pelt and spleen. Tissue concentration on a per kg basis shows that gonads, adrenals, brain and thyroid have significant retention.

(c) *Binding properties of tungsten in milk*

Table 3 shows the results of the ion-exchange studies to determine the binding properties of radiotungsten to milk proteins. The results of this test indicate that most of the radiotungsten in milk is present as an inorganic anion, probably tungstate.

Table 3. Per cent of inorganic tungsten in milk as determined by ion-exchange

Elapsed time (hr)	*In vivo* Mean %	*In vivo* Range %	*In vitro* Mean %	*In vitro* Range %
1	94.5 ± 4.1*	88.9–101.9		
2	96.7 ± 4.3	90.8–102.7		
3			94.5 ± 1.2*	93.0–96.3
4			98.3 ± 2.7	94.6–103.6
5	90.1 ± 2.6	86.8–94.1		
7.5			96.1 ± 2.3	91.4–99.8
8.5	90.6 ± 3.9	84.3–95.8		
11			96.4 ± 2.5	92.3–98.8
12.5	89.0 ± 3.8	81.2–92.9		
15			92.8 ± 1.7	90.1–94.9
25	85.7 ± 2.4	80.8–88.3		
27			95.1 ± 4.2	90.5–104.6
49	86.3 ± 6.3	80.8–95.4		
51			94.3 ± 2.0	90.9–96.8
73	70.3 ± 7.2	61.6–81.6		
75			90.3 ± 1.8	87.8–93.9

* Standard deviation

DISCUSSION

The per cent of administered dose secreted per liter of milk at peak activity was 0.0128 and 0.118 for the groups receiving oral and I.V. doses, respectively. During the 84-hr period after dosing, 80% of the orally administered dose was excreted, with 64% recovered in the feces, 15% in the urine and 0.4% in the milk. In the same time period, only 68% of the administered dose was recovered from the I.V. dosed cows, with 65% recovered in the urine, less than 1% in the feces and 2% in the milk.

A comparison of the results of the multiple oral dose study to the single oral dose study shows the expected similarities in early biological clearance times and secretion rates. These parameters expressed as fraction of intake should be unaffected by the duration of exposure to different isotopes of the same element. The time to reach the peak radionuclide concentration in the milk was influenced by the duration of exposure. The peak radionuclide concentration in milk appeared in 24 hr after the single oral dose of radiotungsten, but in the multiple dose study the peak was not reached until 59 hr after initial administration of tungsten-181. The long biological clearance phases found in the milk for tungsten-181 were not discernible in the single dose tungsten-187 study due to the short half-life of the isotope. Although the amount of activity administered to the cows receiving multiple doses of radiotungsten did not vary significantly from day to day, the curves suggest that a true flat equilibrium curve was not obtained during the period of ingestion, based on concentration. When the total amount of radiotungsten excreted or secreted daily per compartment is calculated the equilibrium is more readily seen. Variations do occur in amounts excreted in the urine and feces as the result of stress factors caused by long confinement in the stalls.

Based on the total body activity the results of the tissue distribution studies show that bone is the major site of retention of tungsten regardless of the mode of administration. This agrees with the findings of other investigations with mice and rats (Ka68; Wa56; Ba60). Comparison of the fraction of the orally administered radiotungsten found in the bone of a calf to that of a nonlactating cow (Table 1) indicates that growing bone has a far greater affinity than mature bone for both the accumulation and the retention of tungsten. On a concentration basis, substantial activity was seen in gonads, adrenals, brain and thyroid. The relatively high activity in the gonads requires further study to assess the potential significance of this finding.

Comparison of the results listed in Tables 1

and 2 indicates that radiotungsten incorporated into milk may be more biologically available since a larger fraction of the radiotungsten was found in the tissue of the calf fed milk from cows ingesting tungsten-181.

In the event of a release of radiotungsten over a dairy farming area it would appear that the milk could be monitored by use of ion-exchange procedures to concentrate the activity for instrumental analysis. The results of these studies indicate that orally ingested tungsten is absorbed in the intestinal tract of the cow. Of the amount absorbed the preferred pathway of excretion for the absorbed tungsten is the urine (14.8% excreted in the urine as compared to 0.441% secreted in the milk). Although milk transfer was relatively limited, this route of exposure for man could be significant, depending upon environmental activity levels. The organ distribution indicates little concentration or long-term retention in edible tissues of the cows. This would allow use of beef animals for meat within a short period of time after the animals receive uncontaminated feed or are removed from a contaminated environment.

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